Ichthyological note – *Note ichtyologique*

© SFI

Received: 14 Dec. 2015
Accepted: 9 May 2016
Editor: A. Gilles

Genetic, morphometric and meristic analyses of first *Acanthurus monroviae* specimens recorded in Maltese waters (Central Mediterranean)

by

Sandra AGIUS DARMANIN*, Noel VELLA & Adriana VELLA (1)

Résumé. – Analyses génétiques, morphométriques et méristiques des premiers spécimens d'*Acanthurus monroviae* répertoriés dans les eaux de l'archipel maltais (Méditerranée centrale).

Cette publication documente l'apparition d'*Acanthurus monroviae* dans les eaux maltaises. Un premier spécimen a été collecté dans ces mêmes eaux le 28 septembre 2013 tandis qu'un second a été capturé le 13 octobre 2015. Cette espèce n'est pas indigène de la Méditerranée mais est native de l'est de l'Atlantique tropical. L'identification des spécimens, grâce à des mesures méristiques et morphométriques, a été confirmée par des analyses génétiques de 2880 pb issues de gènes de l'ADNmt. La présence inhabituelle du chirurgien de Monrovia avait été signalée par des observations et des analyses morphométriques au large des côtes espagnoles en 1981, puis dans l'est du bassin Levantin (Méditerranée orientale), le long des côtes algériennes et tunisiennes ainsi que dans les eaux helléniques. Une observation non confirmée en 2015 dans les eaux maltaises est maintenant certifiée par les analyses détaillées des spécimens collectés.

Keywords. – Acanthuridae - *Acanthurus monroviae* - Mediterranean Sea - Malta alien species - Genetic barcoding - First records.

Human activities, such as shipping, aquaculture, and the opening of the Suez Canal, have led to the introduction of nearly 1000 alien species into the Mediterranean Sea (Katsanevakis *et al*., 2014). It is one of the most threatened area due to human activity (Coll *et al*., 2015) and its increasing seawater temperature is accelerating the establishment of tropical species (Moschella, 2008).

The Monrovia Surgeonfish *Acanthurus monroviae* Steindachner, 1876 is native along the coast of Africa and the Sao Tome, Cape Verde and Canary archipelago (Froese and Pauly, 2015). It was previously reported in the Mediterranean off Marbella southern Spain (Crespo *et al*., 1987), in the eastern basin off Haifa, Israel (Golani and Sonin, 1996), off the coast of Algeria (Hemida *et al*., 2004), off the coast of Tunisia (Ben Souissi *et al*., 2011), off Salamina Island in the Hellenic waters (Batjakas *et al*., 2015) and an unconfirmed record off the Maltese coast (Langeneck *et al*., 2015). Other Acanthuridae in the Mediterranean are the Blue Tang Surgeonfish *Acanthurus coeruleus* Bloch & Schneider, 1801, the Black Tang Doctorfish *Acanthurus chirurgus* (Bloch, 1787) and the Yellow Tang *Zebrasoma flavescens* (Bennett, 1828).

We are reporting in this study the occurrence of an increasing number of specimens of *Acanthurus monroviae* in Maltese waters and we include DNA barcoding for species identification, genetically confirming the first specimen collected.

MATERIALS AND METHODS

The first specimen was collected one mile off St Thomas Bay, Malta (35°51'14.79"N; 14°35'38.87"E) on the 28th September 2013. The second specimen was caught on the 13th October 2015 from the Grand Harbour breakwater (35°54'11.34"N; 14°31'16.66E). Both specimens were caught by spearfishing and kept for scientific analyses by the Conservation Biology Research Group (CBRG). Morphological and meristic analyses of both specimens were undertaken and compared to previous studies and with previously described specimens. The specimens were deposited at the ichthyological collection of the Conservation Biology Research Group laboratory at the University of Malta with the reference CBRG/F.130928/AM001 and CBGR/F.151013/AM001, respectively. Two separate sightings were recorded off the coast of Zurrieq, South East of Malta, on the 11th December 2015 involving several individuals and on the 27th March 2016 involving one specimen (Fig. 1).

To further confirm species identity, tissue samples were collected from the first specimen and preserved in non-denatured 95% ethanol for long-term storage. 30 mg of tissue were digested with Proteinase K and the total DNA was extracted using AccuPrep® Genomic DNA Extraction Kit (Bioneer). PCR amplifications was carried out for: cytochrome c oxidase I gene (COI) using FishF2

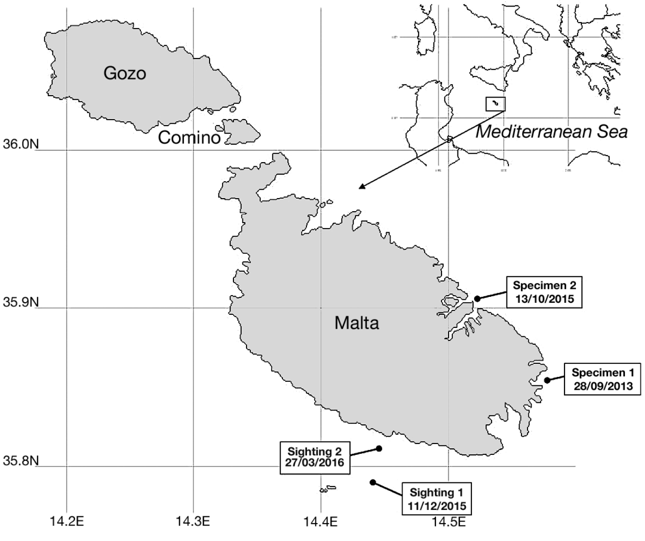

Figure 1. – Locations where specimens were collected and sighted.

---

(1) Conservation Biology Research Group, Department of Biology, University of Malta, Msida MSD2080, Malta. [noel.vella@um.edu.mt] [adriana.vella@um.edu.mt]

* Corresponding author [sandra.agius@um.edu.mt]

Cybium 2016, 40(4): 338-340.

Table I. - Morphometric measurements and meristic counts of *Acanthurus monroviae* specimens caught in Malta and specimens identified off the coast of Tunisia and Hellenic waters. Lengths in mm.

Measurements	Specimen 1	Proportion (%)	Specimen 2	Proportion (%)	Batjakas *et al*. (2015)	Proportion (%)	Ben Souissi *et al*. (2011)	Proportion (%)
Total weight (g)	326.1		230.9		341.6		730.0	
Total length	256.0		235.6		256		379	
Fork length	230.0	89.8 TL	212.0	90.0 TL	223	87.1 TL	335	88.4 TL
Standard length	202.5	79.1 TL	183.2	77.8 TL	194	75.8 TL	283	74.7 TL
Maximum body depth	97.8	38.9 TL	91.5	38.8 TL	104	60.6 TL	128	33.8 TL
Length of dorsal fin base	124.2	61.3 SL	117.4	64.1 TL				
Pectoral fin base	14.0	6.9 SL	13.3	7.3 SL				
Anal fin base	87.3	43.1 SL	80.4	43.9 SL				
Pre-pelvic length	59.4	29.3 SL	57.2	31.2 SL			98	34.6 SL
Pre-anal length	92.4	45.6 SL	73.7	40.2 SL			136	48.1 SL
Head length	56.5	22.1 TL	49.3	20.9 TL	54	21.1 TL	82	21.6 TL
Pre-orbital length	31.0	54.9 HL	28.5	57.8 HL				
Eye diameter	10.4	4.1 TL	11.6	4.9 TL				
Caudal fin length	51.5	20.1 TL	52.6	22.3 TL				
Depth of caudal peduncle	18.1	7.8 TL	16.8	7.1 TL				
Dorsal fin	IX+25		IX+24		IX+26		IX+25	
Ventral fin	I+5		I+5		I+5		I+5	
Anal fin	III+23		III+24		VI+24		III+24	
Pectoral fin	16		16		17			
Lateral line scales	136		127				125	
Gill rakers (on 1st arch)	15		12				12	

and FishR2 primers (Ward *et al*., 2005); cytochrome b (cytb) using GluDG-L14724 and CB3H-15560 primers (Martin and Palumbi, 1993); and 12S to 16S ribosomal RNA genes (12S-16S) using 12SA and 16SA primers (Palumbi, 1996). The amplification for each primer set was performed following the protocols of their respective sources. The PCR products were purified and sequenced via ABI3730xl sequencer using both the forward and reverse primers. The sequences at both nucleotide and amino acid level were analyzed using Geneious v6.1.6 (http://www.geneious.com, Kearse *et al*., 2012). The sequences obtained were deposited in GenBank, with accession numbers KT283583 (COI), KT283584 (cytb), and KT283582 (12S-16S). These sequences were compared to other sequences available in genomic databases using BLASTn.

RESULTS

Morphological identification

The fish specimen morphology, meristics (Tab. I) and colour (Figs 2, 3), are in agreement with the descriptions of the species *Acanthurus monroviae* given by Froese and Pauly (2015), and with the specimens described previously. Their body was rather elliptical, compressed, covered with ctenoid scales and brown in colour with a single elliptical orange-yellow area surrounding a caudal peduncle spine. The first specimen was a male of total length (TL) 256 mm with total body mass of 326.1 g with testes in resting stage. The second specimen having a total length of 235 mm with total body mass of 230.9 g was immature with gonads appearing as very thin strands. Morphometric measurements and meristic counts are given in table I.

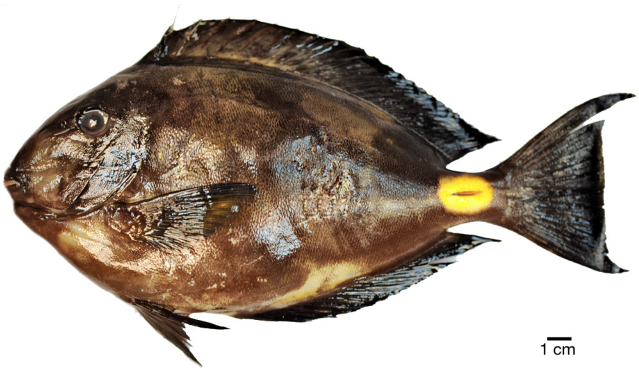

Figure 2. – First specimen of *Acanthurus monroviae* caught off St Thomas bay on 28 September 2013 (Image by A. Vella, CBRG-UoM).

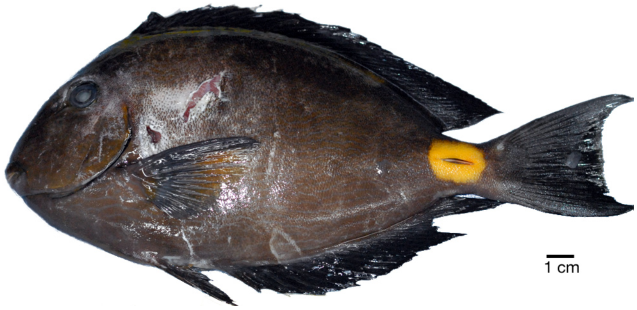

Figure 3. – Second specimen of *Acanthurus monroviae* caught from the Grand Harbour breakwater on 13 October 2015 (Image by A. Vella, CBRG-UoM).

Genetic analyses

A total of 2880 bp were sequenced from the current specimen mtDNA. The sequence lengths obtained were 669 bp, 845 bp and 1366 bp for COI, cytb and 12S-16S, respectively, and each was run via BLASTn to identify sequence matches. All the genes confirmed the genus with high identity matches. At species level the current specimen had 99.7% identity match with KC623666 (Sorenson *et al*., 2013) at COI; and 99.1 to 99.3% matches with HQ234705, HQ234706 (Bernal and Rocha, unpubl. data) and KC623702 (Sorenson *et al*., 2013) at cytb.

The morphometric, meristic and genetic analyses confirm the occurrence of *Acanthurus monroviae* in Maltese waters while providing a first genetic barcode for this alien species found in the Mediterranean Sea.

DISCUSSION

The natural habitat of *Acanthurus monroviae* is rocky and coral bottoms and it also enters the mouth of rivers and lagoons (Debelius, 1997) from 0 to 60 m (Schneider, 1990) where it feeds on zooplankton, phytoplankton and detritus (Diouf, 1996). This species prefers warmer waters from 20°C to 25°C in tropical and subtropical waters (Baensch and Debelius, 1997). A progressive invasion of this species has been observed northwards and eastwards in the Mediterranean Sea and in the northern Atlantic Ocean (Langeneck *et al*., 2015). However, the mechanisms by which this species colonizes areas outside its natural home range remain unknown (Horta e Costa and Gonçalves, 2013). The gradual warming of the Aegean Sea and the Mediterranean Sea facilitates immigration and survival of Atlantic exotic species and may have led to the spread of this species. However, a release to the wild from ornamental fishes trade or from a private aquarium cannot be excluded since the genus is popular in the aquarium trade. The third possibility is the introduction by shipping within ballast water containing larval and juvenile specimens since the first specimen was caught just off bunkering sites in an area with heavy marine traffic and the second specimen was caught just outside a port also with frequent shipping activity. The collection of a second more recent specimen and subsequent sightings also implies that this species has established a population in Maltese waters. This will allow further genetic and phylogenetic analyses of this alien species in the Mediterranean in the near future.

Acknowledgements. – The authors are grateful to Mr. Mark Mercieca, Mr Gordon Pulis and Kingfisher Sport Fishing Association for providing the specimens for analyses and Mathilde Maslin for translating the title and abstract into French.

REFERENCES

BAENSCH H.A. & DEBELIUS H., 1997. - Meerwasser Atlas. 592 p. Mergus Verlag GmbH, Germany.

COLL M., STEENBEEK J., BEN RAIS LASRAM F., MOUILLOT D. & CURY P., 2015. - "Low-hanging fruit" for conservation of marine vertebrate species at risk in the Mediterranean Sea. *Global Ecol. Biogeogr.,* 24: 226-239.

CRESPO J., REY J.C. & GARCIA A., 1987. - Primera cita de *Acanthurus monroviae* Steindachner, 1876 y de *Diodon eydouxii* Brissout de Barneville, 1846 para la ictiofauna europea. *Misc. Zool.,* 11: 271-275.

DEBELIUS H., 1997. - Mediterranean and Atlantic Fish Guide: From Spain to Turkey - From Norway to South Africa. 320 p. ConchBooks.

DIOUF P.S., 1996. - Les peuplements de poissons des milieux estuariens de l'Afrique de l'Ouest : l'exemple de l'estuaire hyperhalin du Sine-Saloum. *Echanges*, 6: 3-4.

FROESE R. & PAULY D., 2015. - *Acanthurus monroviae* summary page. FishBase: http://www.fishbase.us/summary/Acanthurus-monroviae.html (accessed Oct. 2015).

GOLANI D. & SONIN O., 1996. - The occurrence of the tropical West African marine fishes *Acanthurus monroviae* (Acanthuridae) and *Arius parki* (Ariidae) in the Levant. *Aqua, Int. J. Ichthyol.,* 2: 1-3.

HEMIDA F., DIATTA Y., GOLANI D., SOUISSI J.B., GUÉLORGET O. & CAPAPÉ C., 2004. - On the occurrence of the Monrovian surgeonfish, *Acanthurus monroviae* Steindachner, 1876 (Osteichthyes: Acanthuridae) off the coast of Algeria (southern Mediterranean). *Acta Adriat*., 45: 181-185.

HORTA E., COSTA B. & GONÇALVES E.J., 2013. - First occurrence of the Monrovia doctorfish *Acanthurus monroviae* (Perciformes: Acanthuridae) in European Atlantic waters. *Mar. Biodiv. Rec.,* 6: 1-4.

KATSANEVAKIS S., COLL M., PIRODDI C., STEENBEEK J., BEN RAIS LASRAM F., ZENETOS A. & CARDOSO A.C., 2014. - Invading the Mediterranean Sea: biodiversity patterns shaped by human activities. *Front. Mar. Sci.,* 1: 1-11.

KEARSE M., MOIR R., WILSON A. *et al*. [14 authors], 2012. - Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28: 1647-1649.

LANGENECK J., BOYER M., DE CECCO P.G., LUCIANI C., MARCELLI M. & VACCHI M., 2015. - First record of *Acanthurus chirurgus* (Perciformes: Acanthuridae) in the Mediterranean Sea, with some distributional notes on Mediterranean Acanthuridae. *Medit. Mar. Sci.,* 16: 427-431.

MARTIN A.P. & PALUMBI S.R., 1993. - Protein evolution in different cellular environments: cytochrome b in sharks and mammals. *Mol. Biol. Evol*., 10: 873-891.

MOSCHELLA P., 2008. - The new CIESM Tropicalization Programme. *In*: Climate Warming and Related Changes in Mediterranean Marine Biota, CIESM Workshop Monographs n° 35 (Briand F., ed.). 152 p., Monaco, Helgoland, 27-31 May 2008.

PALUMBI S.R., 1996. - Nucleic acids II: the polymerase chain reaction. *In:* Molecular Systematics (Hillis D.M., Moritz C. & Mable B.K., eds), pp. 205-247. Massachusetts: Sinauer Associates.

SCHNEIDER W. (ed.), 1990. - FAO Species Identification Sheets for Fishery Purposes. Field Guide to the Commercial Marine Resources of the Gulf of Guinea. Prepared and published with the support of the FAO Regional Office for Africa. Rome.

SORENSON L., SANTINI F., CARNEVALE G. & ALFARO M.E., 2013. - A multi-locus timetree of surgeonfishes (Acanthuridae, Percomorpha), with revised family taxonomy. *Mol. Phylogenet. Evol*., 68: 150-160.

WARD R.D., ZEMLAK T.S., INNES B.H., LAST P.R. & HEBERT P.D.N., 2005. - DNA barcoding Australia's fish species. *Phil. Trans. R. Soc. Lond*., *Ser. B, Biol. Sci*., 360(1462): 1847-1857.